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"Wiring" of Glucose Oxidase within a hydrogel made with Polyvinyl Imidazole Complexed with $[(Os\text{-}4,4\text{-dimethoxy 2,2'-bipyridine})Cl]^{+/2+}$

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Glucose electrodes based on hydrogels made by crosslinking glucose oxidase and the redox polymer formed upon complexing polyvinyl imidazole (PVI) with $[Os(dmo\text{-}bpy)_2Cl]^{+/2+}$ (dmo-bpy = 4,4'-dimethoxy-2,2'-bipyridine) on vitreous carbon electrode surfaces were investigated. The redox potential of the hydrogels was -69 mV (SCE) and their glucose electrooxidation current reached a plateau at +50 mV (SCE). Urate and acetaminophen were not electrooxidized at this potential at rates that would interfere with the glucose assays. At 32 mM glucose concentration switching of the atmosphere from argon to O_2 reduced the current only by 5%.

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"WIRING" OF LACTATE OXIDASE WITHIN A LOW-REDOX POTENTIAL ELECTRON CONDUCTING HYDROGEL

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Abstract

Lactate electrodes based on electron conducting hydrogels made by crosslinking lactate oxidase and the redox polymer formed upon complexing polyvinyl imidazole (PVI) with $[Os(dmo\text{-}bpy)_2Cl]^{+/2+}$ (dmo-bpy = 4,4'-dimethoxy-2,2'-bipyridine) on vitreous carbon electrode surfaces were investigated. The redox potential of the hydrogels was -69 mV (SCE) and their lactate electrooxidation current reached a plateau at +50 mV (SCE). Urate and acetaminophen were not electrooxidized at this potential at rates that would interfere with the lactate assays but ascorbate was catalytically oxidized by the gel. At 6mM lactate concentration switching of the atmosphere from argon to O_2 reduced the current by 40%, showing that the rate of electron transfer from the reduced enzyme to the gel was slow.

Introduction

The specificity of amperometric biosensors depends on the potential at which they are poised and on the electrocatalytic activity of their electrodes. At enzyme electrodes on which substrates are electrooxidized the rate of electrooxidation of some interferants can increase with the oxidation potential. The merit of using, in enzyme electrodes, diffusional

redox mediators of relatively reducing redox potentials having at pH7 standard potentials that are closer to that of the enzyme is well recognized (Cass et al., 1984). Studies have focused on both mediators and small redox polymers that diffusionally shuttle electrons between redox centers of redox enzymes and metal or carbon electrodes (Gregg, Heller, 1991; Schuhmann et al., 1991). Because they form fast reversible and chemically stable redox couples, 2,2'-bipyridine (bpy) complexes of osmium$^{2+/3+}$ have been studied in this context. Of these, the tris-4,4'-dimethoxy-2,2'-bipyridine complex has a redox potential of 225 mV and is a particularly effective mediator (Zakeeruddin et al., 1992).

In flow systems and also *in-vivo*, diffusional mediator based sensors can usually not be used because of leaching of the mediator. Here it is advantageous to "wire" the enzyme, i.e. complex it with a redox polyelectrolyte to form a water soluble adduct and crosslink the adduct on the electrode surface (Gregg, Heller, 1990). The crosslinked redox polyelectrolyte network swells in water to form a hydrogel to which the enzyme is covalently bound. Because the hydrogel conducts electrons through electron transfer between its polyvinyl pyridine or polyvinyl imidazole bound $[Os(bpy)_2Cl]^{+/2+}$ redox centers and because it is permeable to water soluble substrates and products, the current densities and sensitivities of the resulting electrodes can be high (Heller, 1992; Aoki, Heller, 1993; Rajagopalan et al., 1994). Earlier it was shown that electrooxidizable interferant-caused currents were reduced upon shifting the redox potential of the hydrogel to a more reducing potentials and poising the electrodes at a lower potential. Thus, the potentials were decreased by complexing osmium bis-2,2'-bipyridine chloride $[Os(bpy)_2Cl]^{+/2+}$ to imidazole nitrogens of poly N-vinyl imidazole (PVI)

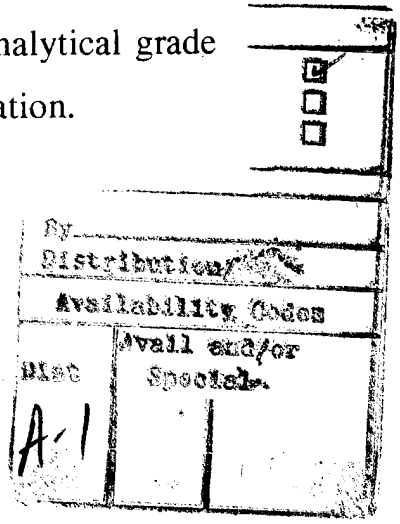
rather than to pyridine nitrogens in polyvinyl pyridine (Lever, 1990). The potential was further decreased, and the selectivity against interferants further increased, by using 4,4'-dimethyl substituted 2,2'-bipyridines with which a polymer denoted as PVI-dme-bpy-Os was made (Ohara et al., 1993). Here we show that the bis-4,4'-dimethoxy-2,2'-bipyridine chloride complex of partially methylated PVI, (PVI-dmo-bpy-Os), has a redox potential of -69mV (SCE). Even though this redox potential is only about 0.3V positive of the FAD/$FADH_2$ redox centers of lactate oxidase, the novel polymer effectively "wires" the enzyme. The lactate electrode made with the hydrogel based on this polymer was insensitive to urate and acetaminophen when poised at +50mV (SCE). Ascorbate electrooxidation, still contributed to the current although it only increased the current by 9.6% at 2mM lactate.

Experimental

Chemicals.

Lactate oxidase (LOx) (Genzyme) 28.0 units/mg., Poly(ethylene glycol) diglycidyl ether (PEGDGE) (Polysciences Inc.), ammonium hexachloroosmate (IV) (Johnson Matthey), were used as received. 1-vinylimidazole (Aldrich) was distilled prior to use. Poly (1-vinylimidazole) PVI was prepared as reported (Chapiro, Mankowski, 1988). Lactate solutions were prepared from a stock solution (1M in phosphate buffer solution). All other chemicals including analytical grade solvents were from Aldrich and used without further purification.

Synthesis.



4,4'-dimethoxy-2,2'-bipyridine (dmo-bpy) was prepared as described (Maerker, Case, 1958) except for modification of the nitration step. 4,4'-dinitro-2,2'-bipyridine-N,N'-dioxide was made by dissolving 10g 2,2'-bipyridine-N,N'-dioxide in 40 mL fuming (1.9 g/cm^3) sulfuric acid, cooled in an ice bath. 30 mL fuming nitric acid (1.48 g/cm^3) was added dropwise and the mixture was kept at 100°C for 4 hours. After cooling to room temperature, the mixture was poured over a small amount of ice, and the product was collected by filtering through a glass frit. The yield was ≈55%. The nitro groups were replaced by the methoxy groups and the N-oxide was de-oxygenated according to reported procedures (Maerker, Case, 1958).

The preparation of the $Os(dmo\text{-}bpy)_2Cl_2$ was carried out under argon. 200 mg of dmo-bpy and 202 mg $(NH_4)_2OsCl_6$ were mixed with 7 mL ethylene glycol, the solution was degassed by bubbling argon and heated for one hour at 200°C. The mixture was then cooled to room temperature and an aqueous solution of sodium dithionite (10mL, 1M) was added. The sodium dithionite reduced the complex, precipitating it. The mixture was stirred in an ice bath for 30 minutes prior to filtering on a fritted glass filter. The yield was 90%.

The $Os(dmo\text{-}bpy)_2Cl_2$ was reacted with the polymer, purified, and partially methylated according to the literature (Taylor et al., 1995). The resulting polymer was analyzed by elemental analysis. Calcd for PVI_{15}-Os-dmo-bpy·$9H_2O$ $C_{99}Cl_2H_{132}N_{34}Os$ C, 52.4; Cl, 3.13; H, 5.88; N, 21.0; Os, 8.39. Found: C, 50.73; Cl, 1.61; H, 6.26; N, 19.04; Os, 7.78.

Electrodes.

3 mm diameter vitreous carbon electrodes were used. These were polished and cleaned using 3 grades of alumina slurry (5, 1, 0.3 micron) with sonication and rinsing between grades. They were tested in phosphate buffer by scanning between the potentials of interest (-0.4 V to 0.4 V vs. SCE) to insure the electrochemistry was featureless. They were prepared by adding 2 μL of the PVI-dmo-bpy-Os (20 mg/mL), 1 μL of GOx (16 mg/mL), and 1 μL of the crosslinker PEGDGE (5.6 mg/mL). The solutions were mixed on the surface of the electrode with the tip of a syringe. The electrode was then placed in an evacuated desiccator for 16 hours prior to use. The resulting dark purple film appeared to be well spread and uniform.

Measurements.

The measurements, unless otherwise noted, were carried out under argon, using a Princeton Applied Research model 273 potentiostat/galvanostat in a 3-electrode cell. Rotating disk experiments were performed using a Pine RDE4 potentiostat, with an MSRX speed controller, an X-Y-Y' plotter, and a VWR 1165 refrigerated constant temperature circulator. All measurements were performed using a 20mM phosphate buffer (pH=7.3) containing 0.1M NaCl, except for measurements in which the pH dependence was followed. In experiments where the pH was varied, 2M solutions of HCl or NaOH were added to the phosphate buffer solution. The rotating disk experiments were run at 21.3°C in 50 mL of phosphate buffer. This cell had a rotating glassy carbon working electrode, a saturated calomel reference electrode (SCE), and a platinum counter electrode, isolated from the bulk solution by a Vycor™ frit. The

rotating disk experiments were performed at 1000 rpm, with a stream of argon purging the solutions, unless otherwise noted.

Results and Discussion

Zakkeeruddin et al reported that the tris $Os^{2+/3+}$ complex of 4,4'-dimethoxy-2,2' bipyridine (dmo-bpy), $Os(dmo\text{-}bpy)_3^{2+/3+}$ accepts electrons at a higher rate from GOx $FADH_2$ centers than other osmium complexes (Zakeerudin et al., 1992), including the parent 2,2'-bis bipyridine complexes originally introduced by Degani and Heller (Degani, Heller, 1989; Pishko et al., 1990). They also reported that the standard potential of this complex was about 350mV more reducing than that of $Os(bpy)_3^{2+/3+}$, and that glucose sensing electrodes made with this redox polymer, when poised at 0.35V(SCE), did not electrooxidize at appreciable rates the common electrooxidizable interferants. For this reason, we investigated the complex of $[Os(dmo\text{-}bpy)Cl]^{+/2+}$ with PVI, (PVI-Os dmo-bpy), as a "wire" of LOx.

Figure 1 shows the cyclic voltammogram of the PVI-dmo-bpy-Os modified vitreous carbon electrode at a 1 $mVsec^{-1}$ scan rate. The oxidation and reduction peaks were separated by 54 mV and the redox potential of the polymer, when crosslinked with PEGDGE on the electrode surface was -53 mV(SCE).

Catalytic electrooxidation (Figure 2) of lactate was observed already at -100 mV(SCE) and the current was close to its plateau at +50 mV(SCE). In electrodes with films containing 40-60 weight% LOx, the 300$\mu A\ cm^{-2}$ current density of glucose electrooxidation was nearly independent of the

enzyme weight fraction (Figure 3). The dependence of the catalytic electrooxidation current of lactate (6 mM) in pH 7.4 phosphate buffer on potential is seen in Figure 4 for an electrode made with 40 weight% lactate oxidase at a loading of 450μg/cm^2 of the PVI-Os-dmo-bpy polymer. The current density at 6mM lactate was greatest at 450μ g cm^{-2} (Figure 5). The apparent Michaelis constant Km of the uncoated electrode at 450μg cm^{-2} loading with 50 weight% LOx, was about 0.6mM lactate (Figure 6). The pH dependence of the electrode is shown in Fig. 7.

Curing of the electrodes at 24°C for 16 hours was inadequate to prevent leaching of the polymer and the enzyme from electrodes rotating at 1000 rpm. The leaching shortened the life of the electrodes. Overcoating with a layer of Nafion™ improved the retention, extending the life of the electrodes rotated at 1000 rpm. (Figure 8).

Overall, the current densities were lower than those observed in LOx electrodes made identically with a more oxidizing (E^0=95mV) complex having methyl rather than methoxy groups in the 4,4' positions of the bipyridine rings. Competition by O_2 for the $FADH_2$ electrons with the $[Os(dmo\text{-}bpy)Cl]^{+/2+}$ centers was severe. The current dropped by 40% when the rotating (1000 rpm) electrode was poised at +50 mV(SCE) in an oxygen-saturated PBS solution containing 6mM lactate, while the related 4,4' dimethyl complex based polymer coated LOx electrode lost only 15% of its current. Overcoating with a Nafion™ film increased the apparent K_m twentyfold to about 20mM lactate, but as expected, simultaneously reduced the sensitivity tenfold (Figure 9). Urate electrooxidation was not observed in the presence of 2mM lactate. Electrooxidation of

acetaminophen was also negligibly slow, not adding to the current even when the acetaminophen concentration was as high as 1mM (Table I). Electrooxidation of 0.1mM ascorbate added only 9.6% to the current at 2mM lactate, increasing the 5.7μA lactate electrooxidation current by 0.55μA.

In summary, the lactate electrode made with PVI-Os-dmo-bpy poised at +50mV(SCE) had advantages with respect to the similar electrode made with PVI-Os-dme-bpy in selectivity against urate, acetaminophen and ascorbate. Its sensitivity toward O_2 was, however, higher.

Acknowledgment

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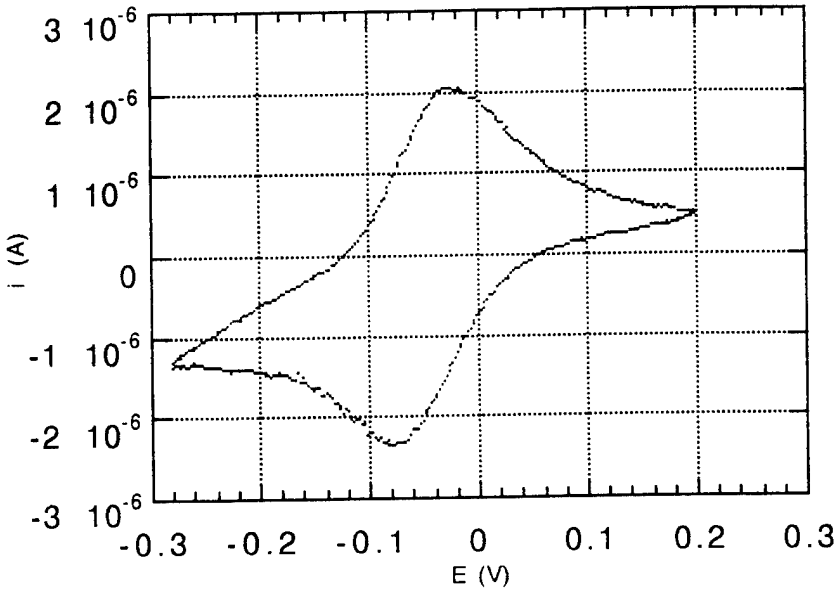

Fig 1. Cyclic Voltammogram of electrode modified with a crosslinked film of PVI_{15}-Os-dmo-bpy; scan rate 1 mV/sec., N_2.

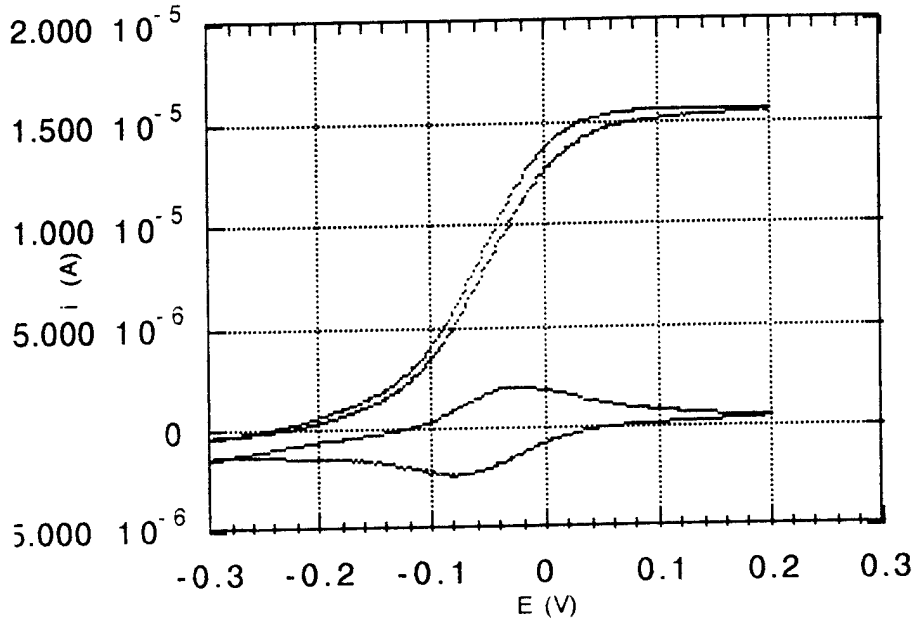

Fig 2. Cyclic voltammogram and catalytic wave with an electrode modified with PVI_{15}-Os-dmo-bpy; scan rate 1 mV/sec., N_2.

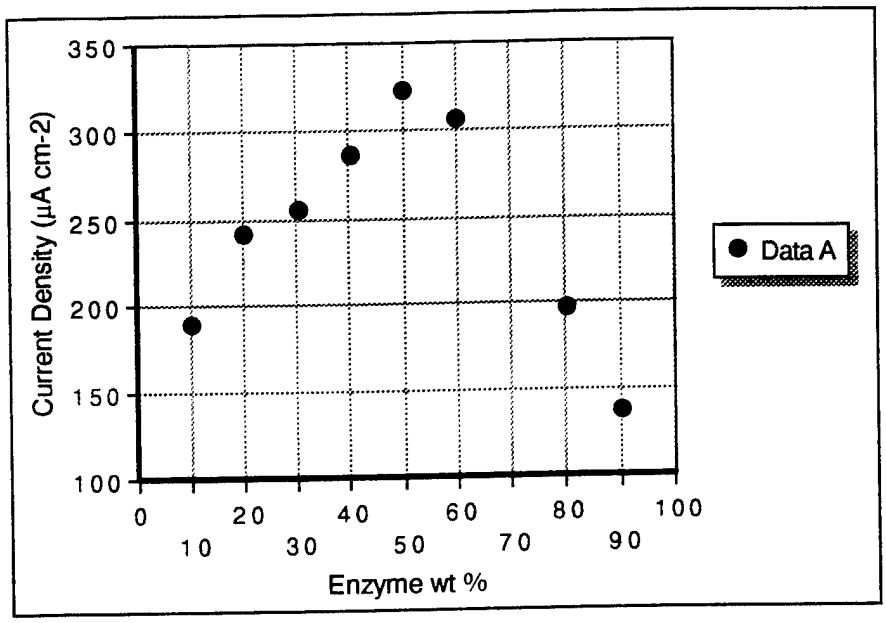

Fig. 3 Dependence of the limiting catalytic current density on the LOx enzyme weight fraction. The data points are the average for two electrodes, 1000 rpm, argon, 6 mM lactate.

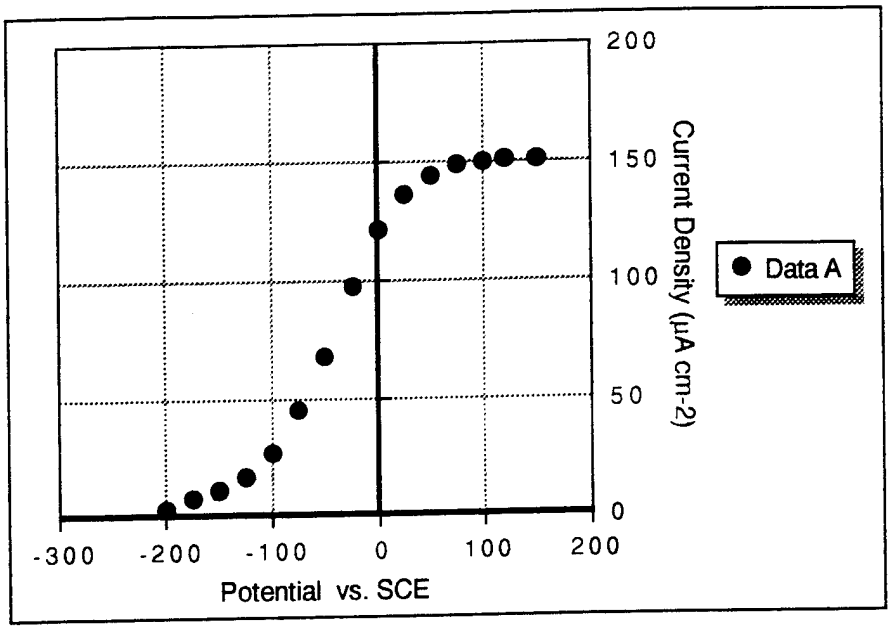

Fig 4. Potential dependence of the steady-state current density for the lactate electrode: 1000 rpm, argon, 6 mM lactate.

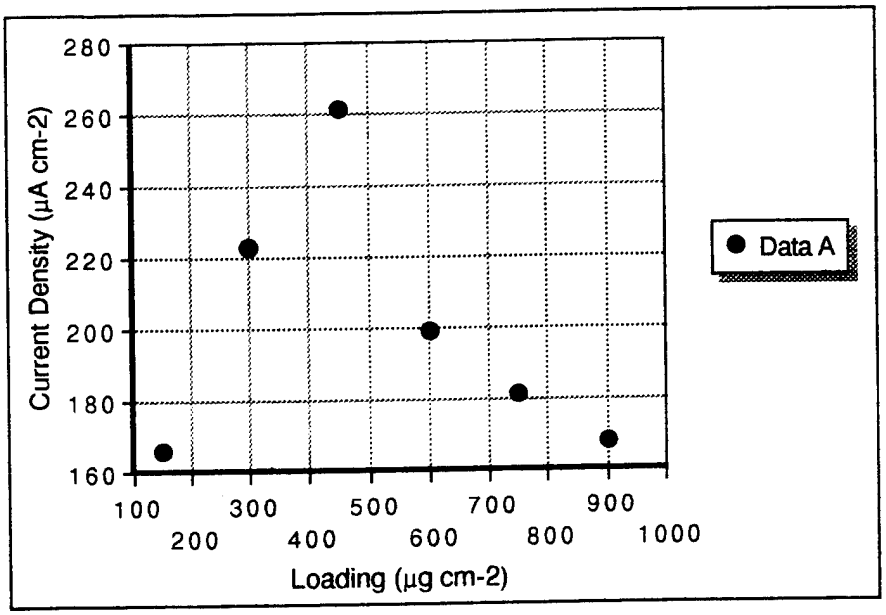

Fig. 5 Dependence of the limiting catalytic current density on the total amount of material coated on the electrode. Lactate sensors were prepared with a fixed wt. % of PVI_{15}-Os-dmo-bpy (65%), LOx (26%), and PEGDGE (9%).

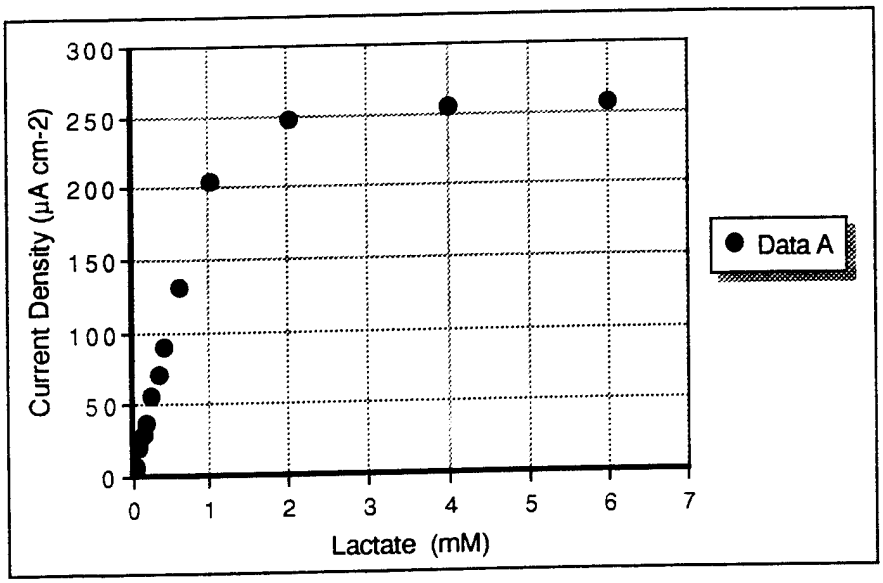

Fig. 6 Steady-state response of the lactate sensor under argon at various lactate concentrations; 1000 rpm.

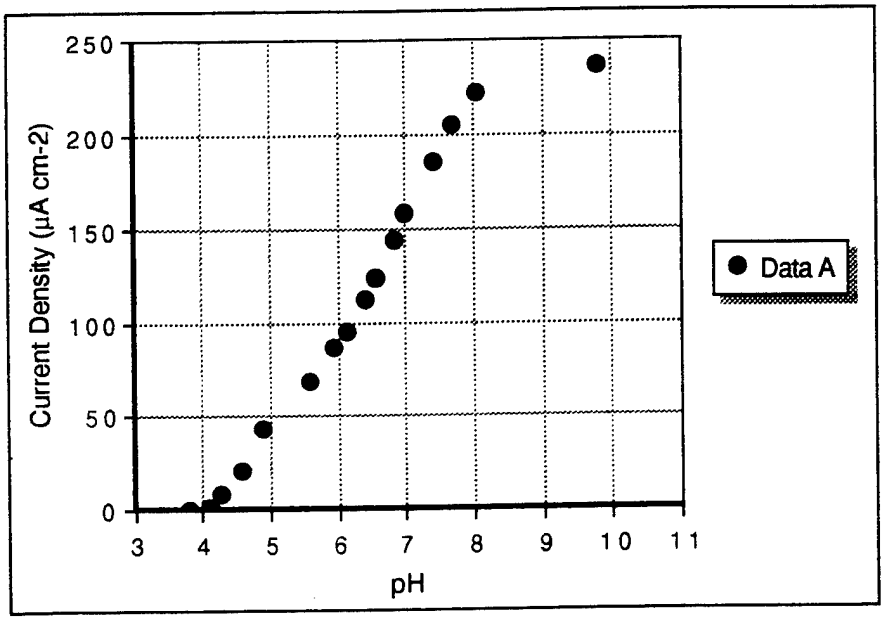

Fig. 7 Dependence of the steady-state current ratio on pH for the lactate sensor: 1000 rpm, argon, 6 mM lactate.

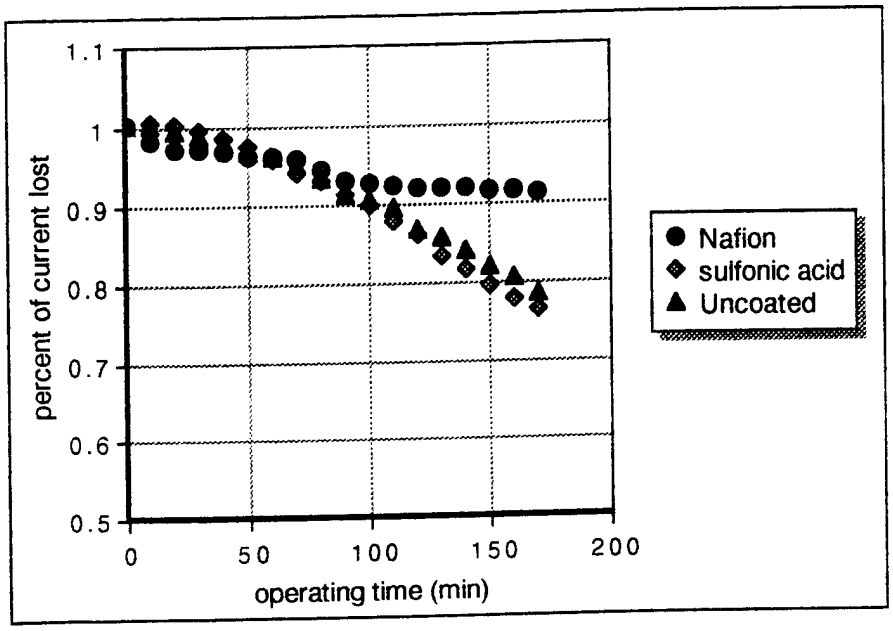

Fig. 8 Dependence of the steady-state current ratio over 170 min. operating time for an uncoated, chlorosulfonic acid treated, and a Nafion™ coated electrode: 1000 rpm, argon, 6 mM lactate.

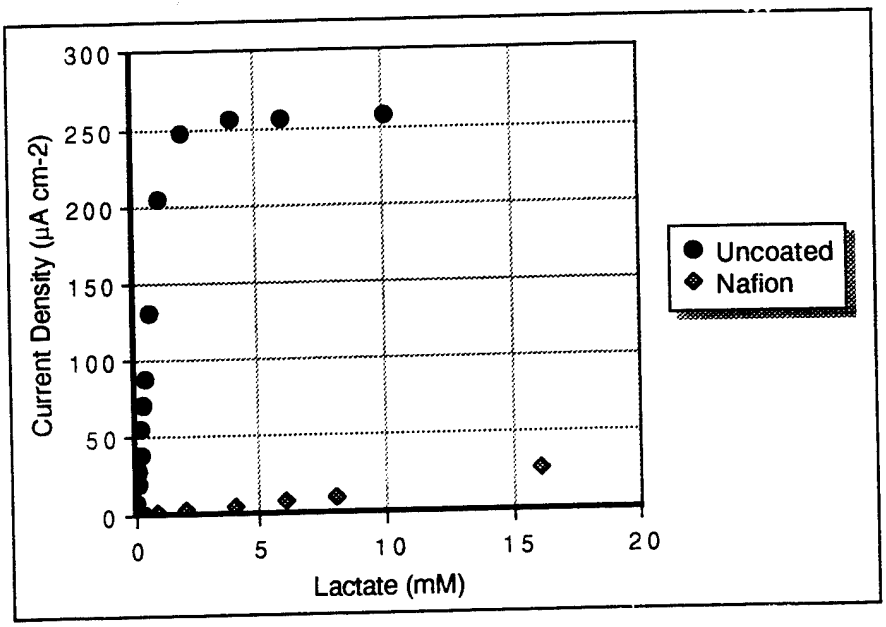

Fig. 9 Steady-state response of an uncoated and a Nafion^{TM} coated electrode: 1000 rpm, argon.

Table I. Electrooxidation Currents of Common Interferants

Interferant	PVI-dme-bpy-Os (μA)	PVI-dmo-bpy-Os (μA)
0.1 mM ascorbate	7.5	2.4
2mM acetaminophen	0.3	0.1
0.5 mM urate	3.0	0

Uncoated electrodes using 20μg of polymer, 20μg LOx, and 4 μg PEGDGE. All currents are in the absence of lactate.